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Attorney's Docket No.: 12557-004001

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant : Andrew Kloek et al. Art Unit : 1652
Serial No. : 10/098,602 Examiner : S. Swope
Filed : March 15, 2002
Title : NEMATODE GS-LIKE SEQUENCES

DECLARATION OF ANDREW P. KLOEK UNDER 37 C.F.R. §1.132

1. I, Andrew P. Kloek, am Senior Director at Divergence, Inc. I received a Ph.D. in Molecular Genetics from Washington University (St. Louis, MO) in 1995 and a B.S in Genetics from Western Kentucky University (Bowling Green, KY) in 1991. I am an inventor on the above-captioned patent application.

2. The *Meloidogyne incognita* protein identified in the above-referenced patent application as SEQ ID NO:2 was identified as a glutamine synthetase (GS)-like protein based, in part, on Pfam analysis.

3. Pfam is a curated database of protein domain families and associated analytical tools. The database and the analytical tools associated with the database are designed to provide more accurate assessment of protein function than can be achieved by simple pair-wise sequence comparisons such as BLAST analysis. Pfam analysis involves comparing a test sequence, here SEQ ID NO:2, to Hidden Markov Model (HMM) descriptions of more than 1800 protein domain families, each of which has multiple representative members. Pfam analysis assigns an S-score for each comparison between the test protein and the HMM of the protein domain family. This S-score is a

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July 15, 2004

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Carrie A. Amonte

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measure of the relatedness of the test protein to the particular protein domain family. The S-score has an associated "e-value". The e-value is a measure of the odds that the particular S-score for the test protein (or an even better S-score) could arise by chance. Put another way, the e-value is the number of hits that would be expected to have a score equal or better than this S-score by chance alone. Thus, the lower the e-value, the lower the likelihood the match between the test protein and the protein domain family is a chance match and the greater the likelihood that the test protein is a member of the protein domain family. Furthermore, the manual curators of individual Pfam protein models set a score threshold called the "gathering threshold" (GA cutoff) which is considered to be trustworthy cutoff above which effectively zero false positives get through.

4. The *M. incognita* gene encoding SEQ ID NO:2 was identified in a search for *M. incognita* ESTs with sequence similarity to the *Mycobacterium tuberculosis* glutamine synthetase gene glnA4 (GenBank® Accession No. F70885) as described in pages 18 to 19 of the specification.

5. When the *M. tuberculosis* glutamine synthetase gene glnA4 (GenBank® Accession No. F70885) is subjected to Pfam analysis, the domain model sequence with the best e-value (i.e., lowest) is the glutamine synthetase domain model sequence. Pfam analysis assigns an e-value of $4.2e^{-89}$ to this comparison. This value indicates that the match is highly unlikely to occur by chance.

6. Pfam analysis of SEQ ID NO:2 revealed that the domain model sequence with the best e-value was the glutamine synthetase domain model sequence, which was assigned an e-value of $1.7e^{-77}$. This value indicates that the match is highly unlikely to occur by chance. This Pfam analysis strongly supports the conclusion that SEQ ID NO:2 is a glutamine synthetase-like enzyme. The Pfam analysis indicates that the predicted glutamine synthetase domain of SEQ ID NO:2 extends from amino acid 115 to 375.

7. A BLAST analysis that compares SEQ ID NO:2 to protein sequences currently in the GenBank® database reveals that nearly all of the proteins that have sequence significantly similar to

SEQ ID NO:2 are identified as glutamine synthetases. This analysis supports the conclusion that SEQ ID NO:2 is a glutamine synthetase-like enzyme. The results of this BLAST analysis is shown below.

Sequences producing significant alignments:	(bits) Value
gil13472698 ref NP_104265.1	glutamine synthetase [Mesorhiz... 384 e-105
gil23500469 ref NP_699909.1	glutamine synthetase family pr... 366 e-100
gil15965966 ref NP_386319.1	CONSERVED HYPOTHETICAL PROTEIN... 361 2e-98
gil15889423 ref NP_355104.1	AGR_C_3883p [Agrobacterium tum... 357 4e-97
gil17988899 ref NP_541532.1	GLUTAMINE SYNTHETASE [Brucella... 350 5e-95
gil45917208 ref ZP_00196362.2	COG0174: Glutamine synthetas... 336 6e-91
gil15609997 ref NP_217376.1	GlnA4 [Mycobacterium tuberculo... 303 7e-81
gil21220110 ref NP_625889.1	putative glutamine synthetase ... 302 1e-80
gil22958785 ref ZP_00006449.1	COG0174: Glutamine synthetas... 301 2e-80
gil41409029 ref NP_961865.1	GlnA4 [Mycobacterium avium sub... 294 3e-78
gil29833267 ref NP_827901.1	putative glutamine synthetase ... 292 9e-78
gil46362694 ref ZP_00225545.1	COG0174: Glutamine synthetas... 278 2e-73
gil46106195 ref ZP_00199871.1	COG0174: Glutamine synthetas... 273 6e-72
gil15865464 emb CAC81335.1	gamma-glutamylisopropylamide sy... 218 3e-55
gil48856911 ref ZP_00311068.1	COG0174: Glutamine synthetas... 211 3e-53
gil24461668 gb AAN62237.1	putative glutamine-synthetase [P... 208 2e-52
gil46915963 emb CAG22734.1	hypothetical protein [Photobact... 182 2e-44
gil28869125 ref NP_791744.1	glutamine synthetase [Pseudomo... 178 3e-43
gil46199267 ref YP_004934.1	glutamine synthetase [Thermus ... 177 6e-43
gil48477605 ref YP_023311.1	glutamine synthetase [Picrophi... 176 1e-42
gil46311445 ref ZP_00212051.1	COG0174: Glutamine synthetas... 172 1e-41
gil46321429 ref ZP_00221806.1	COG0174: Glutamine synthetas... 172 2e-41
gil15616429 ref NP_244734.1	glutamine synthetase [Bacillus... 171 5e-41
gil22972120 ref ZP_00019019.1	hypothetical protein [Chloro... 170 7e-41
gil28872421 ref NP_795040.1	glutamine synthetase [Pseudomo... 168 2e-40
gil23471345 ref ZP_00126675.1	COG0174: Glutamine synthetas... 168 3e-40
gil32422469 ref XP_331678.1	hypothetical protein [Neurospo... 167 3e-40
gil48764498 ref ZP_00269050.1	COG0174: Glutamine synthetas... 167 4e-40
gil26991088 ref NP_746513.1	glutamine synthetase, putative... 167 4e-40
gil48783610 ref ZP_00280062.1	COG0174: Glutamine synthetas... 167 4e-40
gil26991975 ref NP_747400.1	glutamine synthetase, putative... 167 5e-40
gil15597236 ref NP_250730.1	probable glutamine synthetase ... 166 1e-39
gil23105727 ref ZP_00092181.1	COG0174: Glutamine synthetas... 166 1e-39
gil46164186 ref ZP_00136728.2	COG0174: Glutamine synthetas... 166 1e-39
gil26991859 ref NP_747284.1	glutamine synthetase, putative... 165 2e-39
gil23471983 ref ZP_00127311.1	COG0174: Glutamine synthetas... 165 2e-39
gil15595493 ref NP_248987.1	probable glutamine synthetase ... 165 3e-39

4. I hereby declare that all statement made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these

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statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Date: 7/14/04

Andrew P. Kloeck
ANDREW P. KLOECK